

CLAIMS:

1. A method of detecting a difference of one or more nucleotides between a nucleic acid molecule to be tested and a reference nucleic acid molecule, said method comprising subjecting the test nucleic acid molecule to base specific cleavage to generate oligonucleotide fragments, separating the resulting oligonucleotide fragments based on mass by MALDI-TOF MS and/or other equivalent procedure to produce a fingerprint of the oligonucleotide fragments comprising one or more peaks wherein a peak represents the mass of each fragment and identifying an altered peak relative to a reference nucleic acid molecule subjected to the same procedure wherein the presence of an altered peak is indicative of a difference of one or more nucleotides in said tested nucleic acid molecule.
2. A method according to claim 1 wherein the nucleic acid molecule to be tested is amplified by a polymerase chain reaction (PCR) prior to base specific cleavage.
3. A method according to claim 1 or 2 wherein the base specific cleavage results in oligonucleotide fragments of from about 2 bases to about 1000 bases.
4. A method according to claim 3 wherein the base specific cleavage results in oligonucleotide fragments of from about 3 bases to about 500 bases.
5. A method according to claim 4 wherein the base specific cleavage results in oligonucleotide fragments of from about 4 bases to about 100 bases.
6. A method according to ^{claim 1} any one of claims 1 to 5 wherein the base specific cleavage is uracil specific cleavage.
7. A method according to claim 6 wherein the uracil specific cleavage is mediated by uracil-N-glycosylase.
8. A method according to ^{claim 1} any one of claims 1 to 7 further comprising subjecting

fragmentation products to further separation (PSD) to generate a spectrum from decay dependent on the nucleotide sequence of the oligonucleotide.

9. A method according to claim 8 wherein the further separation of fragmentation products is by post source decay (PSD).

10. A computer programme capable of controlling a method of detecting a difference of one or more nucleotides between a nucleic acid molecule to be tested and a reference nucleic acid molecule, said method comprising subjecting the test nucleic acid molecule to base specific cleavage to generate oligonucleotide fragments, separating the resulting oligonucleotide fragments based on mass by MALDI-TOF MS and/or other equivalent procedure to produce a fingerprint of the oligonucleotide fragments comprising one or more peaks wherein a peak represents the mass of each fragment and identifying an altered peak relative to a reference nucleic acid molecule subjected to the same procedure wherein the presence of an altered peak is indicative of a difference of one or more nucleotides in said tested nucleic acid molecule.

11. A method according to claim 9 wherein the nucleic acid to be tested is amplified by PCR prior to base specific cleavage.

12. A method according to claim 9 or 10 wherein the base specific cleavage results in oligonucleotide fragments of from about 2 bases to about 1000 bases.

13. A method according to claim 9 wherein the base specific cleavage results in oligonucleotide fragments of from about 3 bases to about 500 bases.

14. A method according to claim 10 wherein the base specific cleavage results in oligonucleotide fragments of from about 4 bases to about 100 bases.

15. A method according to any one of claims 9 to 13 wherein the base specific cleavage is uracil specific cleavage.

16. A method according to claim 14 wherein the uracil specific cleavage is mediated by uracil-N-glycosylase.

17. A method according to any one of claims 10 to 16 further comprising the further separation of fragmentation products to generate a spectrum from decay dependent on the nucleotide sequence of the oligonucleotide.

18. A method according to claim 17 wherein the further separation of fragmentation products is by post source decay (PSD).

19. An apparatus capable of detecting a difference of one or more nucleotides between a nucleic acid molecule to be tested and a reference nucleic acid molecule, said apparatus comprising means of subjecting the test nucleic acid molecule to base specific cleavage to generate oligonucleotide fragments, separating the resulting oligonucleotide fragments based on mass by MALDI-TOF MS and/or other equivalent procedure to produce a fingerprint of the oligonucleotide fragments comprising one or more peaks wherein a peak represents the mass of each fragment and identifying an altered peak relative to a reference nucleic acid molecule subjected to the same procedure wherein the presence of an altered peak is indicative of a difference of one or more nucleotides in said tested nucleic acid molecule.

20. An apparatus according to claim 19 further comprising further fragmentation separation means to generate a spectrum from decay dependent on the nucleotide sequence of the oligonucleotide.

21. An apparatus according to claim 20 wherein the further fragmentation separation means is post source decay (PSD).

22. Use of MALDI-TOF in the detection of a difference of one or more nucleotides between a nucleic acid molecule to be tested and a reference nucleic acid molecule.

23. Use according to claim 22 further comprising use of PSD to generate a spectrum for

decay dependent on the sequence of an oligonucleotide.

24. A method for identifying and/or locating a mutation in one or more bases in a target nucleic acid molecule, subjecting the test nucleic acid molecule to base specific cleavage to generate oligonucleotide fragments, separating the resulting oligonucleotide fragments based on mass by MALDI-TOF MS and/or other equivalent procedure to produce a fingerprint of the oligonucleotide fragments comprising one or more peaks wherein a peak represents the mass of each fragment and identifying an altered peak relative to a reference nucleic acid molecule subjected to the same procedure wherein the presence of an altered peak is indicative of a difference of one or more nucleotides in said tested nucleic acid molecule.

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25. A method according to claim 24 wherein the nucleic acid molecule to be tested is amplified by a polymerase chain reaction (PCR) prior to base specific cleavage.

26. A method according to claim 24 or 25 wherein the base specific cleavage results in oligonucleotide fragments of from about 2 bases to about 1000 bases.

27. A method according to claim 26 wherein the base specific cleavage results in oligonucleotide fragments of from about 3 bases to about 500 bases.

28. A method according to claim 27 wherein the base specific cleavage results in oligonucleotide fragments of from about 4 bases to about 100 bases.

Claim 24

29. A method according to any one of claims 24 to 28 wherein the base specific cleavage is uracil specific cleavage.

30. A method according to claim 29 wherein the uracil specific cleavage is mediated by uracil-N-glycosylase.

31. A method according to any one of claims 24 to 30 further comprising subjecting fragmentation products to further separation (PSD) to generate a spectrum from decay

dependent on the nucleotide sequence of the oligonucleotide.

32. A method according to claim 31 wherein the further separation of fragmentation products is by post source decay (PSD).